Review of: "Mechanical activation drives tenogenic differentiation of human mesenchymal stem cells in aligned dense collagen hydrogels"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

A very interesting and useful article for understanding the effect of mechanical stimulus on the differentiation of hMSCs. Some points to ponder are given below:

General queries

1. Is tenogenic differentiation in this study due to individual effects of the aligned dense collagen hydrogels and mechanical activation or is it a synergistic effect?
2. A more generic question in such a study and other similar studies is if the mechanical stimulus have a direct effect on the differentiation process or does it produce GFs which then drive the differentiation?
3. Was the applied strain the critical factor or was the tenogenic differentiation a result of haptotaxis because of the ADC hydrogels?

Queries specific to the study

1. Is there an optimal collagen density that drives tenogenic differentiation of the hMSCs? Does this densification or increased alignment augment as a function of time?
2. How does fibrillation of the collagen occur due to the GAE method? Why was the 12G needle chosen over the other needle gauges?
3. Please give details of the tethering process? Does this result in an initial strain on the ADC scaffold/substrate?
4. The painstaking details of this experimental study are much appreciated, although checks on the gene expression at two more intermediate points between the 48 h and 21 days would have greatly enhanced understanding of this process.
5. Why are the mechanical properties of the scaffold held at 20% SS better than the other conditions? Although the strain level is reported at 20%, is this the actual strain experienced by the hMSCs? Also, does this strain change during the experiment dues to viscoelastic deformation of the ADC scaffolds? How is the 20% strain maintained constant?
6. Does the fact that tenogenic differentiation is possibly initiated in the first 48 h of mechanical activation indicate that this process is just that, viz. activation without the need for a continuous mechanical stimulus?
7. In section 3.1.4, authors report that for free floating (FF) scaffolds, scleraxis is also upregulated

(although only 2-fold) while RUNx and Aggrecan are highly upregulated indicating that for osteogenic and chondrogenic differentiation do not require mechanical stimulus or possibly the stimulus to a much lower degree (strain)?

8. What can be the possible reasons for the expression of tenomodulin under cyclic strain (CR) compared to SS?
9. Did the authors observe any increase in cross-linking in the ADCs with increased time?
10. What do the authors mean by tendon-like matrix formation (first paragraph of discussion)?
11. Can the claim that 5-13 wt% collagen vis-a-vis 18-38 wt.% collagen approaches native tendon range be substantiated?

Minor points

1. Please check the y-axis scale in Fig. 3B
2. Please check the x-axis scale in Fig. 6D